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Mutations and modifications support a 'pitted-flexiball' model for α-crystallin

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Abstract

α-Crystallin is renown for resisting crystallization and electron microscopic image analysis. The spatial conformation thus remaining elusive, the authors explored the structure and chaperone functioning by analyzing the effects of site-directed mutagenesis, the properties of naturally occurring aberrant forms of α-crystallin and the influence of chemical modifications. The authors observed that the globular multimeric structure, as well as the chaperoning capacity are remarkably tolerant towards changes and modifications in the primary structure. The essential features of the quaternary structure—globular shape, flexibility, highly polar exterior and accessible hydrophobic surface pockets—support a 'pitted-flexiball' model, which combines tetrameric subunit building blocks in an open micelle-like arrangement.

Keywords: α-Crystallin; Molecular chaperone; Site-directed mutations

1. Introduction

Studying the structure-function relationship of α-crystallin, or any other small heat-shock protein (Hsp), is like solving an equation with two unknowns. Detailed information about their three-dimensional structures is lacking and the mechanisms underlying their chaperone-like functioning largely elude one's understanding. Circumstantial evidence thus must help us to evade this problem. When the amino acid sequence of bovine αA-crystallin became known, in 1973, it was already noticed that the N-terminal region had a pronounced hydrophobic character [1]. It was speculated then that this part of the sequence played a special role in the aggregation behavior of the α-crystallin complex. On basis of intron positions and internal sequence similarities, Wistow [2] later proposed that the N- and C-terminal regions of the α-crystallin subunits constitute two globular domains and an exposed C-terminal extension. Both domains were predicted to consist of two similar β-pleated sheet motifs. Circular dichroism and infra-red measurements indeed reveal that α-crystallin has mainly β-sheet structure [3,4]. A two-domain model is experimentally supported by the biphasic denaturation of α-crystallin subunits upon the addition of chaotropic agents [5,6]. The presence of an exposed C-terminal extension agrees with the observation that the last 20 or so residues of the α-crystallin subunits are especially liable to truncations and modifications (see review [7]). Moreover, according to NMR analyses, the C-terminal eight and ten residues of αA- and αB-crystallin, respectively, occur as a solvent-exposed random coil [8].

Accepting then as a working model a two-domain-with-tail subunit structure, the authors have further explored the possibilities of site-directed and natural mutants of α-crystallin. In addition, chemical modifications were used to gain further insight. The authors attempt here to relate the recent findings to the possible quaternary structure

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of α-crystallin and to its functioning as a chaperone-like protein.

2. The structure of α-crystallin

α-Crystallin, as it occurs in the mammalian lens, is an approximately 800-kDa multimeric complex composed of the two homologous types of 20-kDa subunits αA- and αB-crystallin. Reconstituted homo- or heteromultimeric complexes of αA- and αB-crystallin subunits are somewhat smaller (600-650 kDa) than the native protein [9], but otherwise appear to fully regain their structural and functional integrity [10,11]. Both native and recombinant α-crystallin complexes are characterized by a polydisperse globular morphology, as revealed by electron microscopy [12,13]. Also torus-like [14,15] and ellipsoid [16] particles, as well as chain- [17] and sheet-like assemblages [18,19] have been reported. The complexes have a dynamic structure, being able to exchange subunits [20] and their average size is influenced by the physicochemical conditions [5]. As for the quaternary structure of these dynamical α-crystallin complexes, one can choose from a variety of proposed models (see reviews [7,21]; also [22,23,82]).

Native α-crystallin cannot be found in a monomeric or a low molecular weight oligomeric form. Apparently, the high molecular weight multimeric structure represents the most stable state in terms of free energy. In a first attempt to explore stabilizing intersubunit contacts, the authors tried to disturb multimerization by replacing some hydrophobic residues in bovine αA-crystallin: L37Q in the N-terminal domain; and V72N and F74N in the C-terminal domain [24] (Table 1). Gel permeation chromatography revealed that none of these mutations is really effective in reducing the multimeric size. Another relevant subject in this respect is αA-crystallin of the blind mole rat, which contains as many as nine amino acid replacements as compared with rat αA-crystallin, due to diminished evolutionary constraints [25]. Again, despite the presence of scattered replacements that change the local charge and hydrophobicity, the complexes of recombinant mole rat αA-crystallin display the usual multimeric size (Table 1).

Considering the apparent stability of the multimeric complex towards various point mutations, the authors wondered about the effects of more radical mutational changes. The authors therefore produced recombinant rat αA^{ins}-crystallin [13], which is an alternative splicing product of the αA-crystallin gene in rodents and some other mammals. αA^{ins}-crystallin is identical to the normal αA-crystallin except for an insertion of 23 amino acids, precisely at the demarcation between the N- and C-terminal domains. Surprisingly, even this large insertion does not interfere with multimerization. The αA^{ins}-crystallin subunits become arranged in a stable high molecular weight complex, almost twice the size of normal rat αA-crystallin (Table 1). One might assume that the insertions occur as flexible loops at the surface of the multimeric complex, thus leaving the normal subunit interactions in the interior of the complex intact. However, the authors were unable to resolve the insertion by two-dimensional NMR spectroscopy (RS and JA Carver, unpublished results), which indicates that the conformational freedom of the insertion is restricted. Furthermore, within the insertion there is a tryptophan residue and fluorescence measurements show that this chromophore is localized in a relatively hydrophobic environment [13]. Thus, it seems that the α-crystallin complex has a rather open structure in which a large insertion can readily be accommodated without affecting the overall structural integrity. This is in agreement with the evidence for a non-compact structure coming from physical analyses. Light scattering measurements suggest that less than 40% of the hydrodynamic volume of the α-crystallin complex is actually occupied by the polypeptide subunits [26,27].

Which one of the various proposed quaternary structures of the α-crystallin complex fits best with these experimental observations? Because of the hydrophobic character of the N-terminal domain, it has been proposed that the α-crystallin subunits are amphiphatic and multimerize into a dynamic micellar structure [28]. As pointed out [23,29], such a micelle can have an open structure if there are intersubunit spaces present between adjacent C-terminal domains. Such a micellar arrangement of subunits may explain why the mutations were essentially not effective in disturbing the structural integrity. The formation of a micelle is largely based on hydrophobic interactions and it is conceivable that point mutations, which are relatively minor alterations in the primary structure, do not easily affect a rather aspecific driving force. Also the viability of the αA^{ins}-crystallin complex may be due to the flexibility and non-compactness of the

Table 1
Overview of recombinant αA-crystallins

αA-species	Mutation(s)	Rationale for the mutation(s)	Multimeric size (MDa)[a]	Relative hydrophobicity[b]	Relative chaperone-like activity[c]	Reference
Rat αA	Wild-type		0.69	+	+++	[13]
Rat αA	Residues 64–173	C-terminal domain and extension	0.06	nd	—	[37,48]
Mole rat αA	R12H, E29Q, S51T, F53L, G60C, N123S, R163Q, S172L, S173F	Naturally occurring variant (sequence degeneration due to the loss of visual function)	0.61	+	++	[d]
Rat αA^{ins}	Insertion of LMTHMWFVMHQP-HAGNPKNNPVK between residues 63 and 64 of wild-type αA	Natural mutant (due to alternative splicing)	1.10	++	−/+	[13]
Bovine αA	Wild-type		0.64	+	+++	[24,57]
Bovine αA	Residues 1–63	N-terminal domain	0.8–1.0 (heterogeneous)	nd	—	[37]
Bovine αA	D69S	Replacement of a conserved Asp	0.71	+	++	[24]
Bovine αA	L37Q	Replacement of hydrophobic residue to disturb putative intersubunit contacts	0.64	+	+++	[24]
Bovine αA	V72N	Replacement of hydrophobic residue to disturb putative intersubunit contacts	0.60	+	+++	[24]
Bovine αA	F74N	Replacement of hydrophobic residue to disturb putative intersubunit contacts	0.58	+	+++	[24]
Bovine αA	170APSK173	Replacement in the wild-type 170APSS173 sequence to study the effect of polar residues in the C-terminal extension	0.64	+	+++	[57]
Bovine αA	170ALGKG174	Replacements in the wild-type 170APSS173 sequence to study the effect of polar residues in the C-terminal extension	0.65	+	+++	[57]
Bovine αA	170ALRKG174	Replacements in the wild-type 170APSS173 sequence to study the effect of polar residues in the C-terminal extension	0.65	+	+++	[57]
Bovine αA	170ALDKG174	Replacements in the wild-type 170APSS173 sequence to study the effect of polar residues in the C-terminal extension	0.64	+	+++	[57]
Bovine αA	170ALWKG174	Replacements in the wild-type 170APSS173 sequence to study the effect of polar residues in the C-terminal extension	0.61	+	+	[57]

[a] Multimeric size was determined by gel permeation chromatography.
[b] Hydrophobicity was determined by ANS binding.
[c] Comparable results were obtained with the heat-protection assay, using β_{low}-crystallin as substrate [10] and in the insulin aggregation assay [41].
[d] R Smulders, M van Dijk, S Hoevenaars, R Lindner, JA Carver and W de Jong (manuscript in preparation).
nd Not determined.

micellar structure, the insertion peptides being accommodated in the intersubunit spaces. If α-crystallin has indeed a micelle-like structure, it should be noted that this structure differs from the classical micelles formed by small surfactants such as sodium dodecyl sulphate. First of all, classical micelles are characterized by a critical micelle concentration (CMC). However, for α-crystallin the CMC is controversial because even at very low concentrations this protein occurs as a multimeric complex [30–32]. A second issue that needs to be clarified before fully embracing a micellar structure, is the precise orientation of the subunits in the complex. In classical micelles the apolar parts of the surfactants are largely shielded from the solvent because they are sequestered in the hydrophobic core. In contrast, it is known that at least some of the hydrophobic regions of α-crystallin are solvent-accessible [33,34]. Using the hydrophobic probe bis-ANS the authors could now demonstrate that in αB-crystallin these hydrophobic regions are located in the N-terminal domain [35].

An essential feature of a micellar structure is that subunits occupy equivalent positions. There is indeed considerable evidence that this is the case for the subunits in an α-crystallin complex [28,36]. There is, however, also good evidence that the complex has tetrameric building blocks. The authors earlier demonstrated that the putative C-terminal domain-with-tail of αA-crystallin, expressed as a recombinant protein, folds properly and assembles into tetramers, probably as dimers of dimers [37]. Moreover, in the presence of 1% deoxycholate, αA-crystallin complexes dissociate into 80-kDa tetramers, without altering the secondary or tertiary structure [38]. Also, during renaturation of denatured α-crystallin at very low concentrations, tetrameric intermediates are observed [38a]. These data suggest that there are rather specific interactions, notably between the C-terminal domains of the subunits. Indeed, site-directed spin labeling studies on rat αA-crystallin indicate that residues I110 to E113, located in a β-strand in the C-terminal domain, are involved in intersubunit contacts [38b]. Based on tetrameric building blocks, a rhombododecahedral model, with twelve 4-fold symmetrical faces, has been worked out [39]. The tendency to form tetramers, combined with the required open pockets in a flexible globular structure, leads us to propose the 'pitted-flexiball' model, as depicted in Fig. 1 and further explained in its legend. This model combines the new experimental evidence with features of the previously proposed micellar-like aggregate [28], the rhombic dodecahedron [39] and the open micellar model [23]. The tetrameric building blocks, the explicit assumption of a two-domain-with-tail rather than a two-domains-with-connecting-peptide subunit structure, structurally equivalent and exchangeable αA- and αB-subunits and the presence of freely flexing polar C-terminal extensions are among the major features distinguishing the pitted-flexiball from the open micellar model.

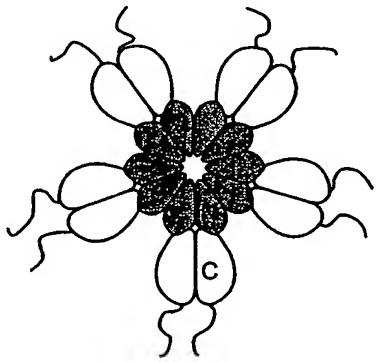

Fig. 1. The pitted-flexiball model for α-crystallin. The αA- and αB-crystallin subunits occupy equivalent positions and can assemble in every possible ratio. Each subunit is composed of two domains. The N-terminal domains (gray) constitute the center of the complex, while the larger C-terminal domains (open) are spaced at the periphery, providing a polar pitted-coat around a hydrophobic core. The hydrophilic C-terminal extensions flex freely from the surface. Specific interactions between the C-terminal domains of the subunits result in the formation of tetrameric building blocks, probably arranged as dimers-of-dimers. Hydrophobic interactions between the N-terminal domains provide a micelle-like cohesion between the building blocks and allow a great flexibility to the size and shape of the complex. Crucially, parts of the hydrophobic regions of the N-terminal domains are solvent exposed in the spaces between the C-terminal domains. These surface pits are accessible for unfolding substrate proteins. Also the phosphorylation sites located in the N-terminal domains of αB-crystallin and Hsp25/27 are in this manner accessible for kinases. The model leaves open whether the hydrophobic pits are separate or form contiguous valleys and whether the tetrameric protrusions of C-terminal domains contact each other in a regular pattern or are irregularly spaced over the surface. The latter seems more likely, considering the problems to obtain satisfactory electron microscopic images. The 5-fold radial symmetry in this figure is arbitrarily chosen, the arrangement of tetrameric building blocks may well be less regular than depicted. The major features distinguishing this model from other 'open micellar' models [23,82] are the oligomeric building blocks, the prominent role of the flexible C-terminal extensions and the presence of accessible hydrophobic regions for substrate binding on the N-terminal domains.

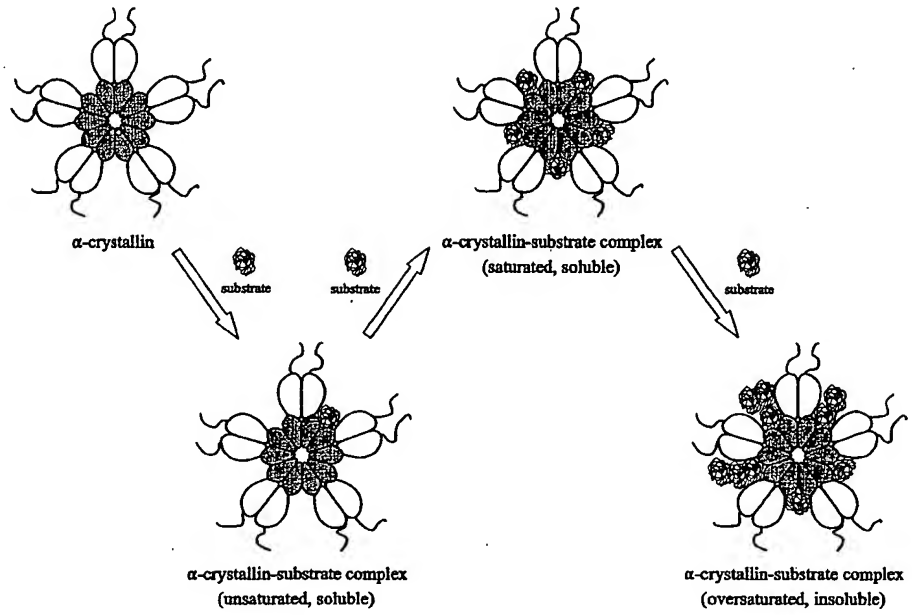

Fig. 2. Proposed scheme for the chaperone-like activity of α-crystallin. The α-crystallin complex is assumed to have a defined number of substrate binding sites, which may vary in accessibility depending on the physicochemical conditions. Hydrophobic regions of the N-terminal domains are essential parts of these sites and are accessible in the surface pits of the flexiball model. The hydrophilic C-terminal extensions and surface charge of the C-terminal domains provide the solubilizing capacity to counteract the increasing hydrophobicity when denaturing proteins interact with the binding sites, until saturation is reached. When the number of unfolded substrate molecules further increases, exceeding the available binding sites or solubilizing capacity of the complex, the oversaturated aggregate becomes insoluble. In this scheme a small substrate is depicted, but larger unfolding proteins can equally well be accommodated. For comparison, the C-terminal domain of the α-crystallin subunits is an approximately 10-kDa structure.

3. The chaperone-like activity of α-crystallin

The chaperone-like behavior of α-crystallin and related small Hsps refers to their ability to bind denaturing or destabilized proteins in vitro, thereby suppressing aggregation and precipitation of these proteins [10,40]. The substrate specificity of α-crystallin is rather low, it can suppress the aggregation of a variety of proteins, including non-lenticular proteins such as alcohol dehydrogenase, citrate synthase and insulin [10,40–42]. Proteins captured by α-crystallin are characterized by a very low degree of unfolding [43] and may be in the molten-globule state [44]. The chaperoning capacity may vary, depending on the type of unfolding substrate and the type of α-crystallin subunit or mutant. The higher the chaperone-like capacity, the more unfolded substrates can be bound before the aggregate of α-crystallin and substrate becomes insoluble [13,45]. Using the pitted-flexiball as a model, the chaperoning process can be envisaged to occur as schematically depicted in Fig. 2 and described in its legend.

Site-directed mutagenesis has been used by other groups to explore the chaperone-like activity of α-crystallin. For αB-crystallin, the results range from complete abolishment of chaperone activity in the F27A mutant [46] to the absence of any effect on complex size and chaperone function when the 43-kDa maltose-binding protein is fused to the N-terminus [47]. How do the αA-crystallin mutants, together with other evidence, support and refine the chaperoning mechanism proposed in Fig. 2? It is understandable that the tetrameric C-terminal domains of αA-crystallin by themselves, lacking the hydrophobic binding sites, are devoid of chaperone-like activity [48]. On the other hand, the recombinant N-terminal domains alone form very large and poorly soluble aggregates, equally unsuitable for the chaperoning task [37]. It thus is obvious that the two domains in concert are required for a viable chaperone. As mentioned above, the presence of the 23-residue insert peptide in αA^{ins}-crystallin does not affect the structural integrity of the complex. It does, however, result in a three to four times lower capacity to bind unfolding β_{low}-crystallin as compared with normal αA-crystallin [13]. The insert peptide, located between the N- and C-terminal domain, apparently hinders the access of unfolding sub-

strates to the binding sites in the surface pits, either sterically or by interacting itself with the hydrophobic sites.

Initial stages of protein unfolding are usually characterized by an increased exposure of apolar surfaces. This suggests that the chaperone-like binding mechanism must largely be based on hydrophobic interactions with unfolding intermediates [43,44,49]. As stated before, α-crystallin itself is even in its native conformation a hydrophobic, yet highly soluble, protein. It is known that slight perturbation of the conformation of α-crystallin, by heat or chaotropic reagents, results in an increase of hydrophobicity as well as substrate binding capacity [50,51]. Taken together, this indicates that the presence of solvent-exposed hydrophobic regions in α-crystallin is a prerequisite for substrate binding. However, in addition to hydrophobicity there must be other parameters that modulate the efficiency of α-crystallin to suppress aggregation of substrate proteins. This is evident from Table 1, which shows that the absolute hydrophobicity of αA-crystallin mutants, as determined by ANS-binding studies, is not directly proportional to their chaperone-like capacity. Differences in conformation and polarity are likely to be additional contributing factors.

Proteins that expose a large number of hydrophobic sites often have a high tendency to form insoluble aggregates [52]. However, in spite of its hydrophobic nature, α-crystallin is very soluble [53,54] and its complex size remains unchanged over a very broad range of concentrations [32]. In addition, the α-crystallin complex is able to remain soluble when loaded with large amounts of denaturing substrate proteins, even though this process further increases the hydrophobicity of the complex [44,49]. It thus appears that α-crystallin is very efficient in counterbalancing the exposure of hydrophobic surfaces. This must be effected by pronounced polar interactions. Indeed, there are various indications that surface charges are extremely important for the functional integrity of α-crystallin. First of all, there is a strong electrostatic repulsion between the highly negatively charged α-crystallin complexes [26,27]. This strongly favors solubility and counteracts the formation of inproductive aggregates. Secondly, both αA- and αB-crystallin appear to display an extreme avoidance of changes in charge during their evolution [55]. Thirdly, the authors have found that the chaperone-like activity of bovine αA-crystallin is not affected by various uncharged amino acid replacements, but decreases when a single aspartate is substituted by a serine [24] (Table 1). Finally, the phosphorylated forms of αA- and αB-crystallin have, in this study, a slightly higher chaperoning capacity than the unphosphorylated forms, while blocking positive charges of lysine side chains by carbamylation does not diminish, but rather tends to improve the chaperone activity [56](Table 2).

To explore charge effects on chaperone-like activity in more detail, the authors have studied a number of αA-crystallin mutants in which charged and hydrophobic residues were inserted into the C-terminal extension [57]. It appeared that introduction in this flexible tail of additional charged residues, be it negative or positive, has only minor effects on the substrate binding capacity. However, introduction of a hydrophobic tryptophan gave an immobilization of the C-terminal extension and a concomitant decrease of functional activity. The authors therefore postulate that a hydrated flexible C-terminal extension is indeed required for increasing the capacity of αA-crystallin to form soluble complexes with denaturing proteins, but that the precise nature of the polar residues in this extension is less important. The importance of the hydrophilic extension as such is also obvious from truncation studies. Proteolytic C-terminal truncation of α-crystallin reduced chaperone capacity [58,59], as does removal of 17 C-terminal residues in recombinant human αA-crystallin [60]. Such truncation also results in increased complex size [60], as had already been noticed in the earlier days of α-crystallin research [17].

Considerable support for the importance of a strong negative charge for the α-crystallin subunits comes from recent modification studies with citraconic anhydride (Table 2). It has earlier been observed that citraconylation of α-crystallin, which abolishes the positive charges of lysine residues and adds negative carboxyl groups instead, results in gradual dissociation of the complex [61]. The authors have now found that this acidification is associated with considerable increase of chaperone activity, without noticeably affecting the secondary structures, as assessed by CD measurements. Even the highly modified, very low molecular weight forms of citraconylated αB-crystallin retain their increased chaperoning capacity. This suggests that such highly negative

Table 2
Overview of post-translationally modified α-crystallins

Modification	Multimeric size (MDa)[a]	Influence on chaperone-like activity[b]	Reference
Phosphorylation of αA or αB[c]	0.60	0/+	[56][d]
Carbamylation of αA or αB[e]	0.50–0.60	0/+	[56][d]
Oxidation (H_2O_2) of native α-crystallin	0.60–0.70	—	[56][d]
Early glycation of native α-crystallin[f]	0.65	0	[56][d]
Early glycation of αA or αB[f]	0.60	0	[56][d]
Late glycation of native α-crystallin[g]	0.60–0.70	—	[56][d]
Late glycation of αA or αB[g] (subunit crosslinking)	0.60–0.65	—	[56][d]
Citraconylation of αA or αB[h]	0.04–0.70	+	[d]

[a] Multimeric size was determined by gel permeation chromatography.
[b] Chaperone-like activity, as compared with the unmodified α-crystallin, were determined with the heat-protection assay and the insulin aggregation assay (left and right of slash, respectively, if different).
[c] Phosphorylated αA- and αB-crystallin from bovine lens.
[d] M van Boekel (manuscript in preparation).
[e] Levels of modification could not be determined; modified subunits positioned on the far acidic side of iso-electric focusing gel.
[f] Approximate modification level of 1 mole sugar/mole subunit.
[g] More than 90% subunit crosslinking, mainly dimerization.
[h] With increasing levels of modification, the complex size first slightly increases, followed by a drastic decrease.

subunits behave like quasi-detergent molecules, where the hydrophobic N-terminus is still capable to bind unfolding proteins, while the polar C-terminus and extension provide the solubilizing power.

In the pitted-flexiball structure, like in the open micellar model for α-crystallin [23,62] the hydrophobic N-terminal domains are still accessible for substrates via the spaces between the C-terminal domains. It should be noted, however, that in the open micellar model the N-terminal domains are not essential as substrate binding regions, i.e. substrate proteins are thought to interact electrostatically with the polar C-terminal domains [29]. In the pitted-flexiball model these interactions are not excluded, but the major importance of the C-terminal domains and extensions lies in the provision of overall solubility. That is, the C-terminal parts of α-crystallin subunits are assumed to constitute a sort of stabilizing interface between the solvent and the hydrophobic binding sites in the interior of the complex. This explains how α-crystallin efficiently combines the exposure of hydrophobic surfaces with a very high solubility. Furthermore, it explains why α-crystallin binds early unfolding intermediates [43,44] rather than proteins which are already in a state of gross unfolding [45,49]. Unfolded and therefore very hydrophobic proteins may be unable to reach the substrate binding sites because they cannot pass through the polar interface at the surface of the complex.

There have been various attempts to locate the binding sites for unfolding proteins on the α-crystallin complex. Important in this respect is the finding that bound substrates, i.e. spin-labeled peptides, do not appear to be clustered, but spread over the complex [41]. The experiments with the hydrophobic probe bis-ANS showed that binding of this probe to the N-terminal domain diminishes the ability to suppress the aggregation of insulin [35]. This supports the notion that the N-terminal domain of αB-crystallin has indeed the ability to bind substrate proteins. Hydrogen-deuterium exchange studies on αB-crystallin likewise pinpointed sequences in the N-terminal region as substrate interaction sites [63]. However, the latter study also found evidence for interacting sites in the C-terminal domain of αA-crystallin and the same was reported for a more distant relative, pea Hsp25 [64]. This, again, makes it likely that both domains are involved in guiding and binding the substrates in the surface pits [62].

An intriguing aspect of the chaperone-like activity of α-crystallin and other small Hsps is the stoichiometry of substrate binding. Denatured γ-crystallin can bind to α-crystallin up to a 1:1 monomer ratio [45] and for rhodanese prevention of aggregation occurs at a ratio of 33 enzyme molecules per molecule of α-crystallin [65]. For other substrates, the binding ratios are often less clear. In the pitted-flexiball model there is no reason to expect a binding of substrates in a

precise 1:1 monomer ratio. Each pit will be lined by hydrophobic surfaces from at least two, but probably four or, depending on the flexibility, even more N-terminal domains. At saturation, all sorts of binding ratios can be expected, depending on size, structure and exposed hydrophobic areas of the substrate.

4. Epilogue

During protein evolution, tertiary structure remains much better conserved than the primary structure. Therefore, a good structural model of α-crystallin should in essence be valid for the related small Hsps as well. The C-terminal domain being the characteristic conserved feature of the superfamily [66], would suggest that the tetrameric or dimeric building block is likely to be a common quaternary structure principle. A dimer structure has indeed been proposed as the minimum cooperative subunit of mammalian Hsp25 [67], although similar calorimetric studies suggest a monomer for α-crystallin [68]. Hsp12.6 of *Caenorhabditis elegans* [69] is the first reported case of a monomeric sHsp, which may relate to its greatly reduced N-terminal domain and lacking C-terminal extension. The trimer-of-trimeric structure of Hsp16.3 of *Mycobacterium leprae* [70] requires considerable flexibility to reconcile with a dimer-of-dimeric model and suggests that fundamental differences in symmetry may occur in more distant members of the superfamily. The N-terminal domain, which in the sHsp superfamily is much more variable in length and in sequence, readily provides the flexibility to accommodate in the pitted-flexiball model the whole range of multimer sizes reported for the various sHsps, ranging from 9 to 40 or more monomers. It provides, moreover, a ready explanation for the fact that certain small Hsps, like mammalian Hsp25 and p20, can reversibly dissociate into low molecular weight oligomers, while others, like αB-crystallin, do not [71]. Such differences in N-terminal sequence and dissociation behavior may, in turn, explain why certain small Hsps do enter the nucleus and others not, or less so [72,73].

The structural model and working mechanism of α-crystallin, as proposed in Figs. 1 and 2, may provide a reasonable explanation for its in vitro chaperone-like properties. It also explains why single amino acid replacements are unlikely to completely abolish chaperone activity: there are no active sites as in an enzyme or residues required for ATPase activity. The very nature of the chaperone-like binding process assumes the involvement of aspecific hydrophobic interactions, presumably between more extended regions. But what about the more intricate properties and in vivo functions ascribed to α-crystallin and other small Hsps? The present model does not directly offer suggestions how bound substrates can be released and refolded, either spontaneously [40,74] or in cooperation with Hsp70 [64,75]. Similarly, it leaves unanswered how α-crystallin and other sHsps can be involved in actin and desmin polymerization [76–78], in cellular signaling [79] and apoptosis [80,81]. As for the ultimate structure-function model of α-crystallin, the contest is still open.

Acknowledgements

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